ORIENTATION OF β-CAROTENE AND RETINAL IN LIPID BILAYERS

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1. Introduction

The function of carotenoids in membranes of photosynthetic bacteria and plants has been intensively investigated [1–4]. In animals certain carotenoids such as β-carotene fullfil an essential physiological function as precursors of vitamin A. By enzymatic oxidation β-carotene is converted to retinol which is further oxidized to retinal i.e., vitamin A.

The positioning of carotenoids in membranes is far from clear [5,6]. No conclusive experiment has been performed showing the orientation of any carotenoid in lipid bilayers or biological membranes. Here, we have used polarized light spectroscopy and studied the orientation of β-carotene and retinal in different types of lipid bilayers.

2. Experimental

The chromophores all-*trans* β-carotene and all-*trans* retinal were from Sigma and EGA-chemie. The lipids 1-oleoyl-*sn*-glycerol (monoolein) and polyglycerol monolaurate were from Aktieselskabet Grindstedvaerket Aarhus. 1-Octanoyl-*sn*-glycerol (monooctanoin) was synthesized at 'Syntestjänst, Chemical Centre (Lund). 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOL) was obtained by acylation of *sn*-glycero-3-phosphocholine with the fatty acid anhydride as in [7]. The mixtures of the chromophore and lipid were prepared by dissolving appropriate amounts of the components (molar ratio of lipid/chromophore $\approx 10^3$) in chloroform, which was then removed by freeze drying. The lamellar liquid crystalline phases of the various lipids were obtained by adding suitable amounts of water and were then left for ~2 days to reach equilibrium.

The samples were protected from light with aluminium foil during the preparation. The lamellar phases were macroscopically aligned between quartz plates [8,9]. Polarized absorption spectra were recorded with a Varian, Cary 219 supplemented with sheet polarizers (HNP'B, Polarizers (United Kingdom) Ltd).

3. Results and discussion

Lipid bilayers containing small amounts of β-carotene or retinal were macroscopically aligned parallel with the quartz plates. The optical axis of such a uniaxially anisotropic sample coincides with the normal (the so-called director) to the quartz plates. By shining polarized light with polarisations forming the angles ω and 90° ($\perp$) with respect to the director the absorbances A_ω and $A_\perp$ are measured. From the dichroic ratio $D_\omega = A_\omega/A_\perp$, information about the molecular orientation of the chromophore can be obtained. An order parameter, S can be calculated [10] from the equation:

$$D_\omega = 1 + 3\,S(1 - S)^{-1} n^{-2} \cos^2\omega \tag{1}$$

where n denotes the refractive index of the lamellar phase. The order parameter S describes the average orientation of the chromophore (the electronic transition dipole moment fixed in the molecule) with respect to the director. For example when $S = -0.5$ the transition moment is perpendicular to the director and for $S = 1$ it is parallel to it. For an isotropic solution S is equal to zero.

Table 1 shows the dichroic ratios obtained together with the calculated order parameters. Assuming that

Table 1
The dichroic ratio, $D_\omega(\omega = 45°)$, and the calculated orderparameter, S, of β-carotene and retinal in different lamellar liquid crystalline samples at 22°C

System	β-Carotene		All-*trans*-retinal	
	Dichroic ratio $(D_\omega)^a$	Order parameter (S)	Dichroic ratio $(D_\omega)^b$	Order parameter (S)
1-Octylmonoglyceride –water; 70–30% (w/w)	0.78	–0.46 (1.46)	1.26	0.28 (1.48)
Polyglycerol monolaurate–water; 70–30% (w/w)	0.89	–0.18 (1.45)	1.31	0.31 (1.46)
1-Oleoylmonoglyceride –water; 10–90% (w/w)	0.94	–0.09 (1.47)	1.20	0.23 (1.51)
Dioleoylphosphatidylcholine–water; 80–20% (w/w)	0.87	–0.22 (1.45)	1.21	0.23 (1.47)

[a] At 460 nm; exp. accuracy of D_ω is ±0.01; [b] at 380 nm; exp. accuracy of D_ω is ± 0.01

The refractive indices used are given within the parentheses

the electronic transition moments are parallel to the long axes of the chromophores the following conclusions can be drawn:

1. β-Carotene tends to be oriented with its long axis perpendicular to the lipid acyl chains for all the lamellar systems studied.

2. Retinal, on the other hand, is preferentially oriented with its long axis parallel with the hydrocarbon chains.

Our studies clearly show that independently of the bilayer thickness (varying from ~16–40 Å), β-carotene (~25 Å long) never spans the lamella. This is not surprising from a physico–chemical point of view since β-carotene is a hydrophobic molecule and therefore prefers to reside within the apolar interior of the membrane. In monooctanoin which forms the thinnest bilayers studied here the order parameter even approaches –0.5 (table 1). This means that the long axes of the β-carotene molecules are almost parallel to the lamellar surface. Also in thick bilayers the β-carotene molecules tend to adopt this orientation although the ordering is less pronounced than in the thin bilayers. These findings are in contrast to [5,6] (cf. fig.3 of [6]).

Retinal, being amphiphilic, is solubilized in the bilayer with the hydrophilic group anchored at the water–bilayer interface having the long hydrophobic tail directed towards the center of the lamellae. It can therefore be expected that carotenoids containing hydrophilic groups at one or both ends may be oriented in a similar way as retinal in the bilayer and they may consequently span the bilayer. Such studies will be reported in a forthcoming paper. The idea that carotenoids can act as light protecting pigments [4] is supported by our findings, since absorption of light impinging on the membrane is more efficient when the transition dipoles of the light collector are parallel to the plane of the membrane as found for β-carotenne. Experimental indications show that the long axes of carotenoids in spinach chloroplasts are parallel to the photosynthetic membranes [11].

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